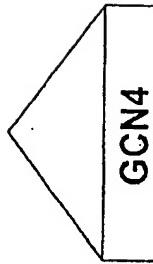
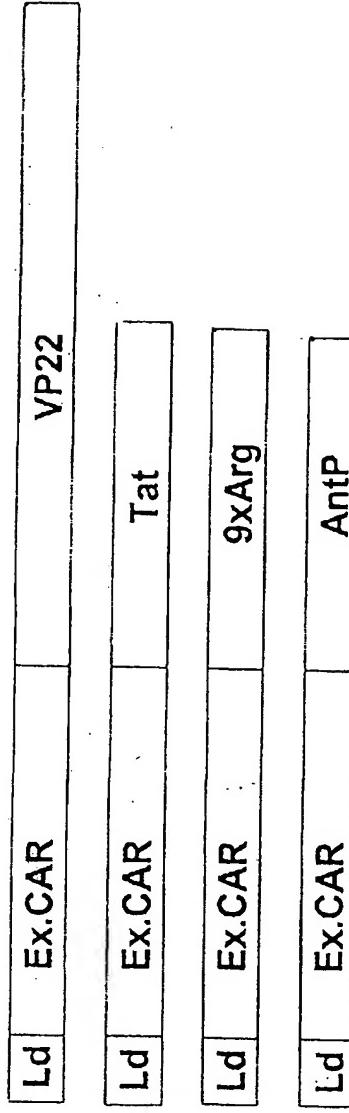
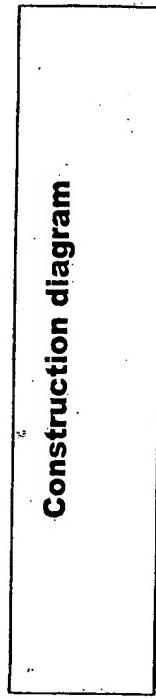


U.S. Patent Application No. 10/583,249  
Inventor(s): Stephan KUBICKA  
Filed: June 16, 2006 Art Unit: Not yet assigned  
For: MEDIZINISCHE HOCHSCHULE HANNOVER  
Attorney Docket: Q95566  
Sughrue Telephone No.: 202-293-7060  
REPLACEMENT DRAWING Fig. 1

**Figure 1**

**Construction diagram**



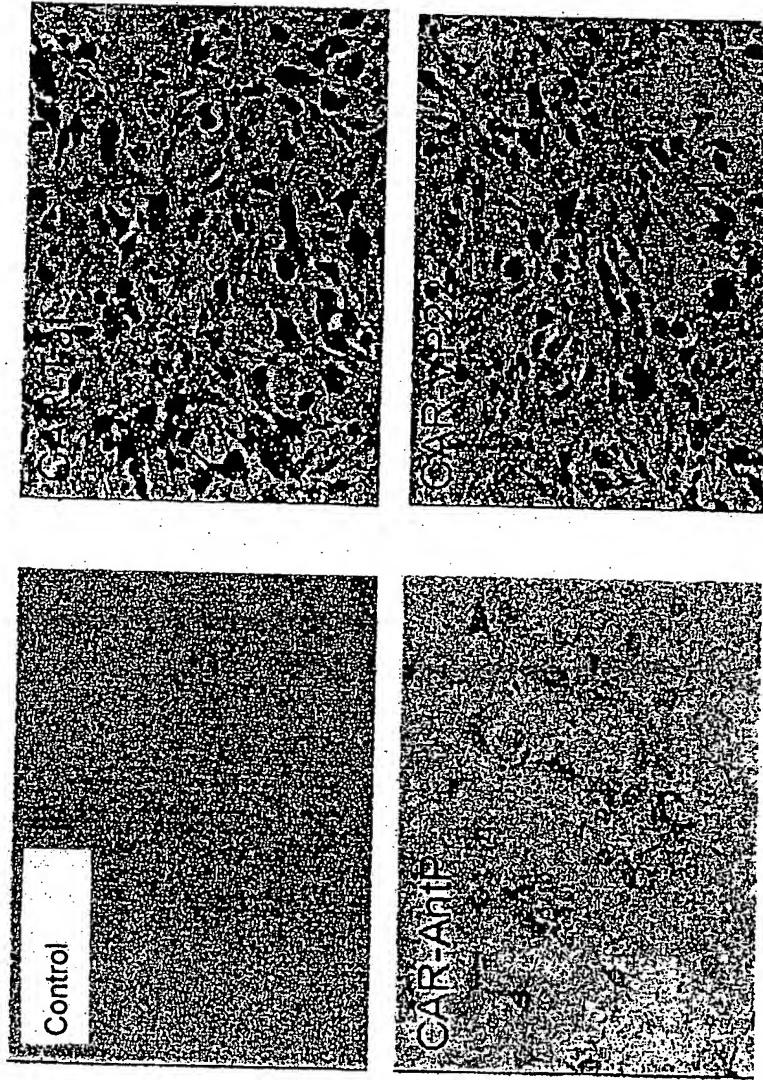
**Legend:**

- Ld** = natural leader sequence of the coxsackie adenovirus receptor for synthesis of the protein into the endoplasmic reticulum
- Ex.CAR** = extracellular domain of the coxsackie adenovirus receptor
- GCN4** = optional insertion of an oligomerization domain (here GCN4 as an example) for possible intensification of the CAR/fibre knob affinity

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REPLACEMENT DRAWING Fig. 2

**Figure 2**

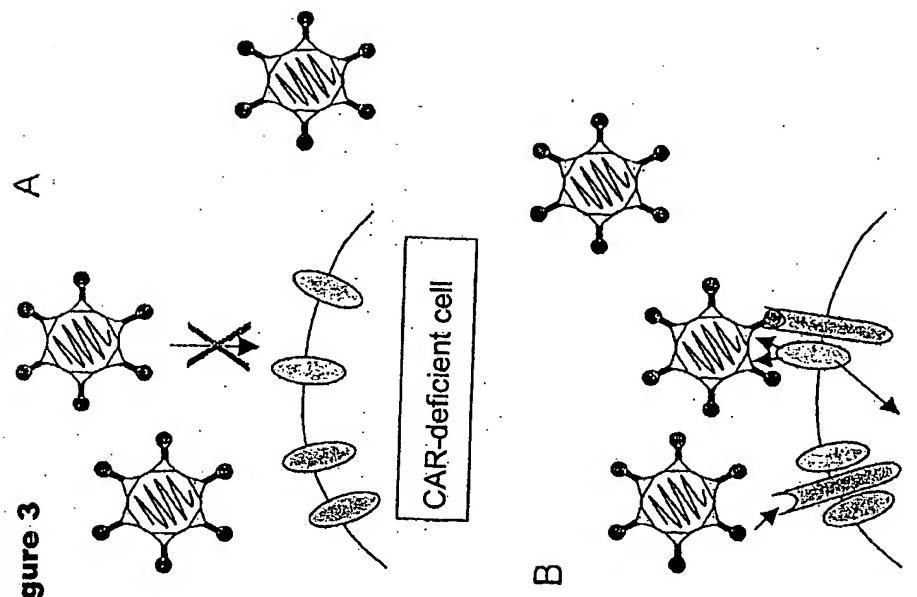


**Fusion proteins from the extracellular domain of the Coxsackie adenovirus receptor and basic peptides or VP22 increase the adenoviral infection of CAR-deficient NIH3T3 fibroblasts.**  
293 cells were transfected with expression constructs for the fusion proteins shown in the figure (pBluescript as a control). After 36 h the supernatants of the cell layer were removed and mixed with LacZ-transgenic adenoviruses (Ad-LacZ). Thereafter, NIH3T3 fibroblasts were infected with this mixture. The multiplicity of infection (MOI) here was 10. After 48 h the infected NIH3T3 cell layer was analysed for  $\beta$ -galactosidase expression by blue staining by X-gal substrate conversion in order to demonstrate the viral infection

▷ = penton base  
 ○ = fibre knob

○ = integrin

 = CAR  
 = CAR fusion protein



**Figure 3**

CAR fusion protein bound to the adenoviral hexon protein directs and sticks the particle to the cell membrane. Integrins consequently bind to the adenoviral penton protein as a signal for the internalization of the particle via endocytosis

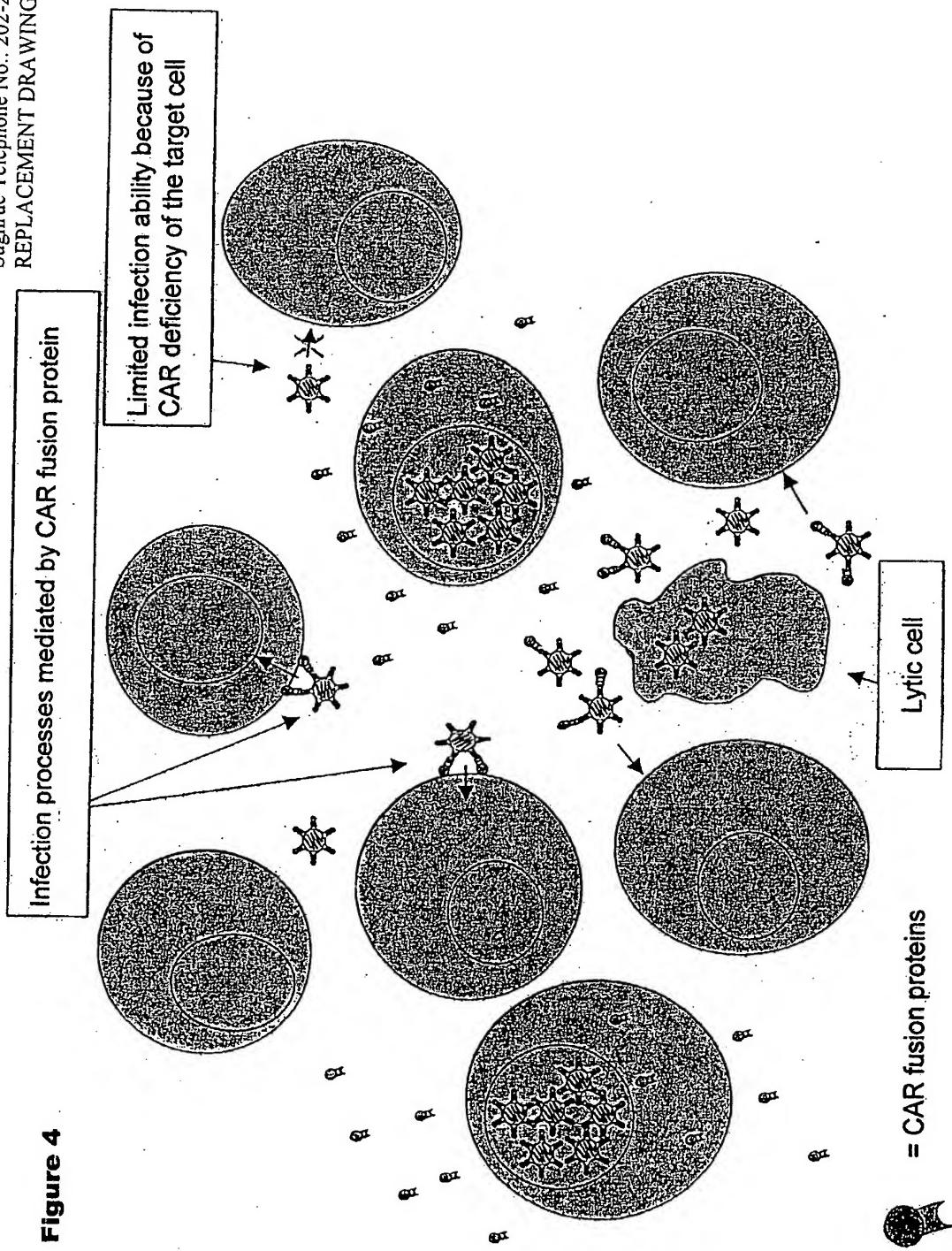
**CAR-deficient cell in the presence of CAR fusion proteins**

CAR binds the hexon protein of the viral particle, penton-bound integrin consequently gives the signal for internalization via endocytosis

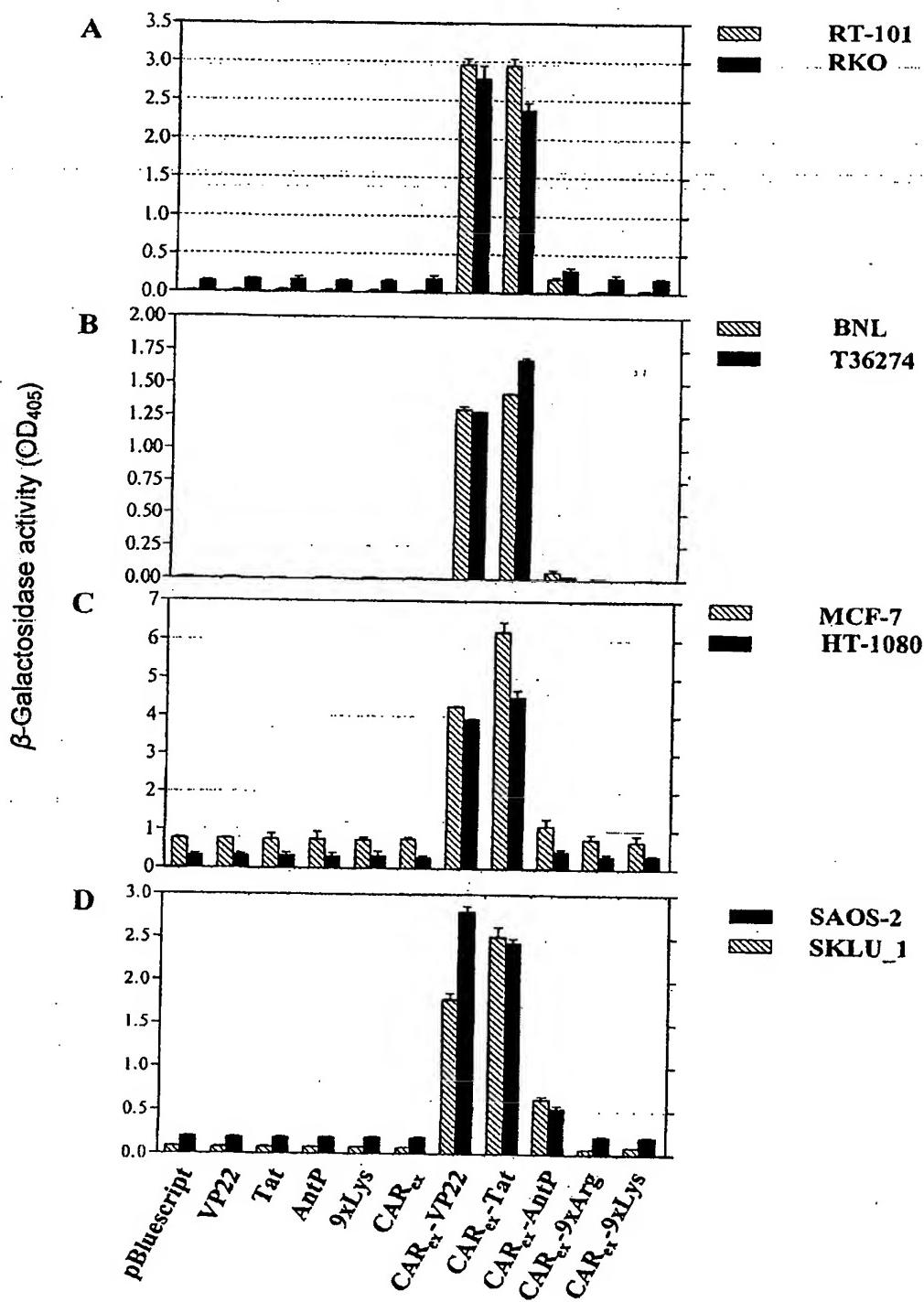
**CAR-expressing cell**

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REPLACEMENT DRAWING Fig. 4

**Figure 4**



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**Fig. 5: Infection efficiency of various cell lines**

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REPLACEMENT DRAWING Fig. 6

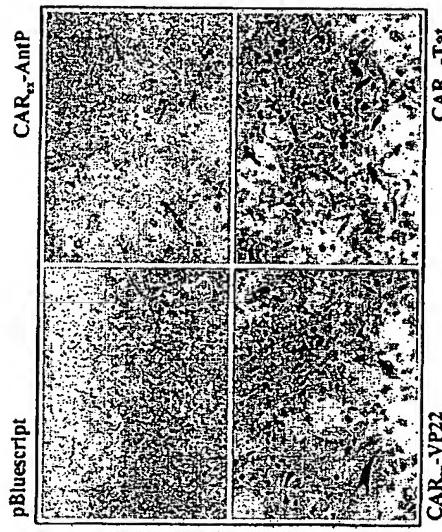
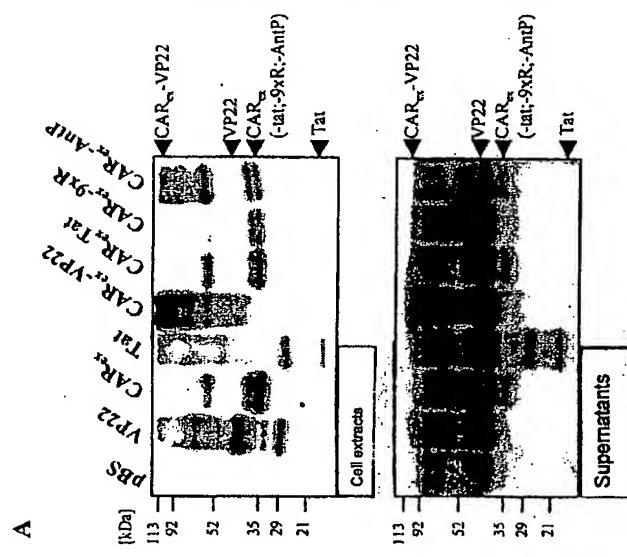
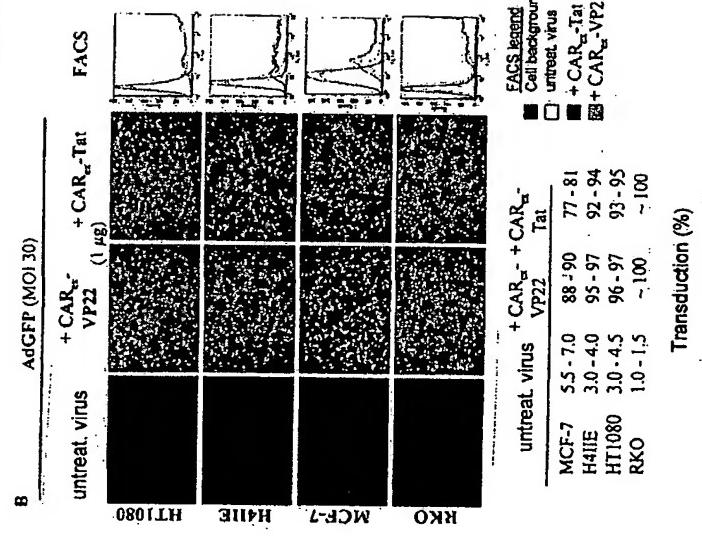


Fig. 6

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	(MOI 10; 4 h infection)		
	untreat. virus	+ CAR <sub>α</sub> -Tat	+ CAR <sub>α</sub> -VP22
SAOS-2	0.5 - 2	45 - 55	50 - 65
SKLU-1	~0	55 - 65	65 - 70
MCF-7	2 - 3.5	40 - 50	50 - 60
BNL	~0	15 - 20	15 - 20
H4IIE	2 - 3	50 - 55	30 - 40
HT1080	1 - 2	60 - 70	65 - 70
RKO	0.5 - 1.5	65 - 75	60 - 70
RT101	~0	15 - 25	15 - 25
T-36274	~0	35 - 45	35 - 40

av. transduction (%)



Transduction (%)

	untreat. virus	+ CAR <sub>α</sub> -VP22	+ CAR <sub>α</sub> -Tat
MCF-7	5.5 - 7.0	88 - 90	77 - 81
H4IIE	3.0 - 4.0	95 - 97	92 - 94
HT1080	3.0 - 4.5	96 - 97	93 - 95
RKO	1.0 - 1.5	~100	~100

Fig. 7

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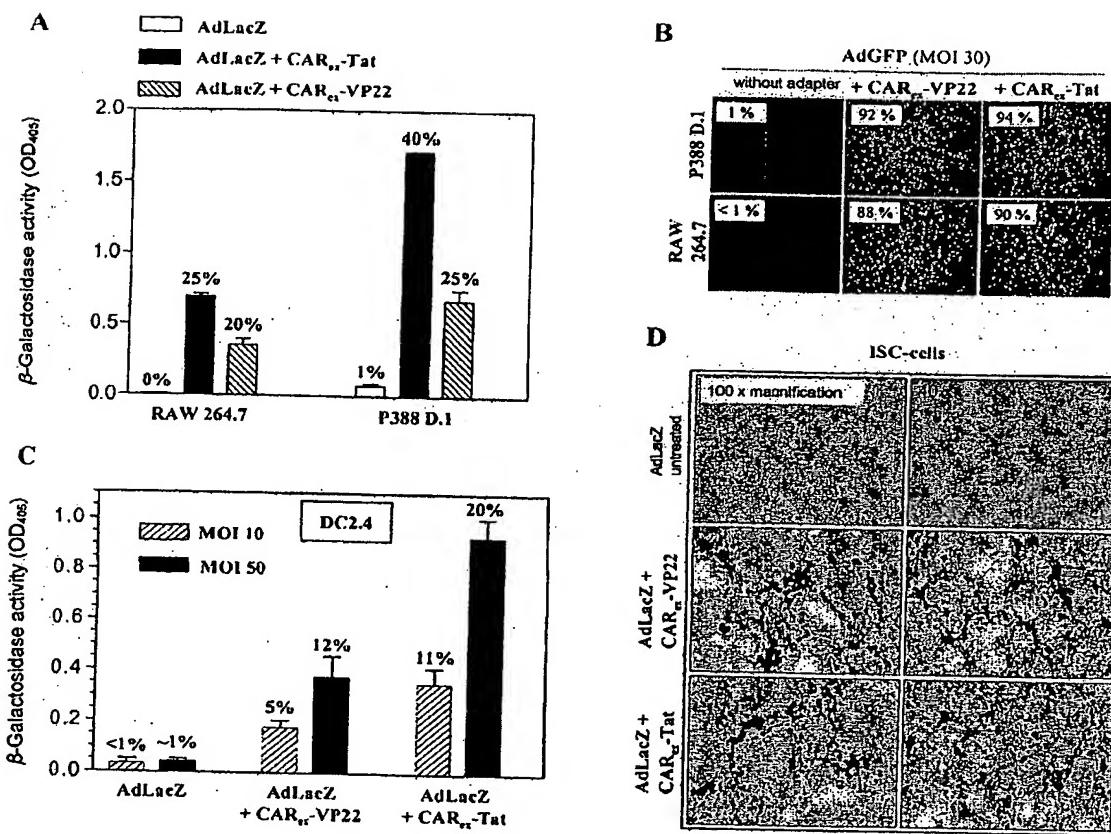


Fig. 8

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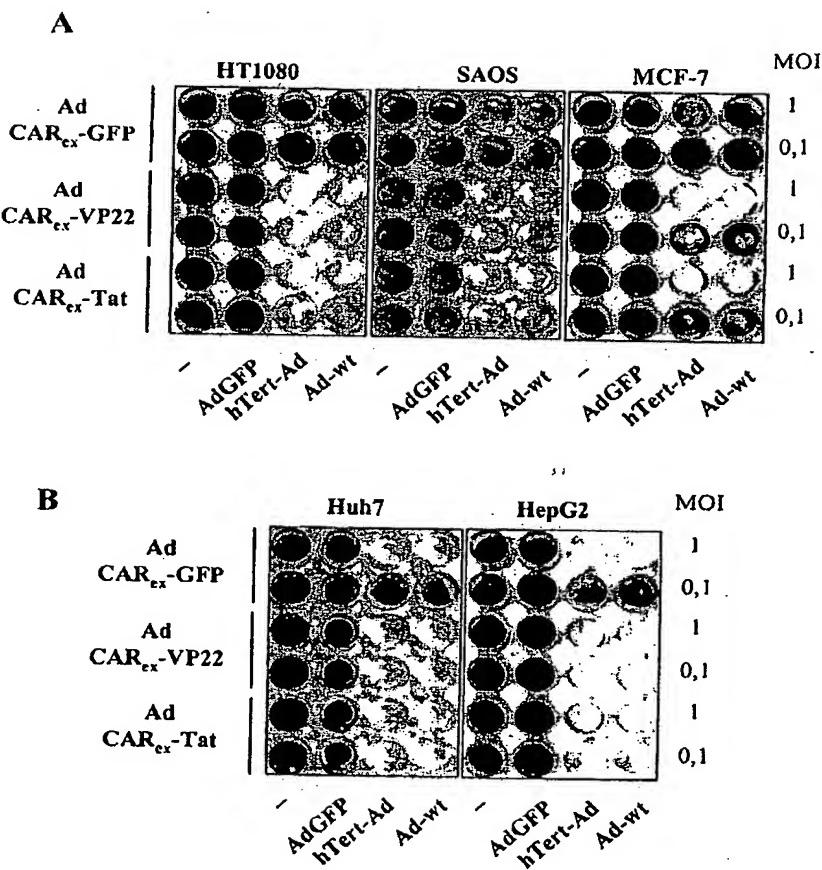


Fig. 9

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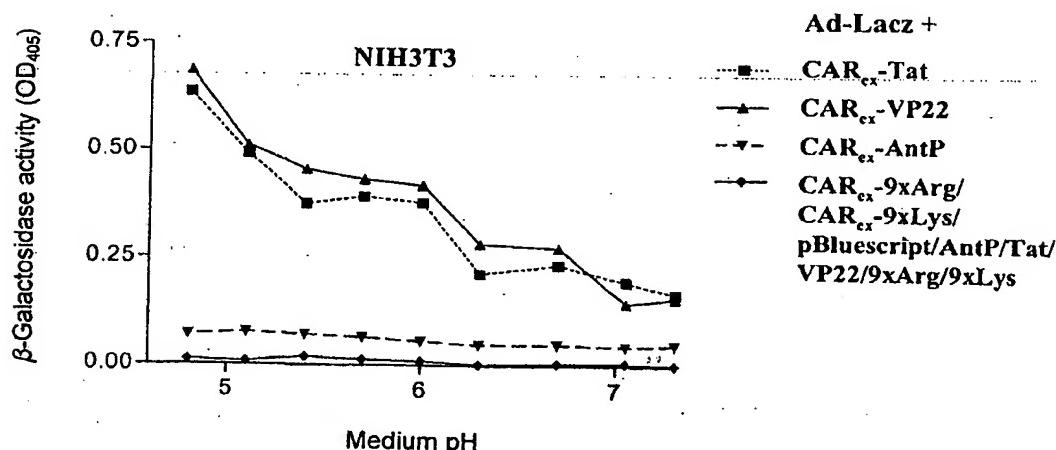
REPLACEMENT DRAWING Fig. 10



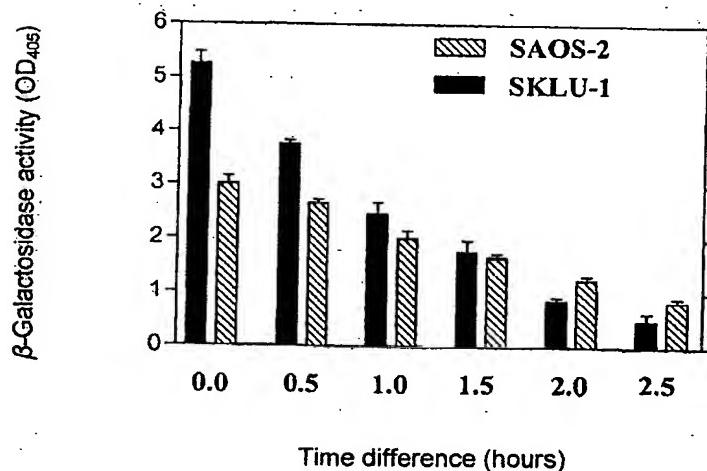
Fig. 10

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**Fig. 11: Influence of the pH**



**Fig. 12: Influence of the time delay**



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